

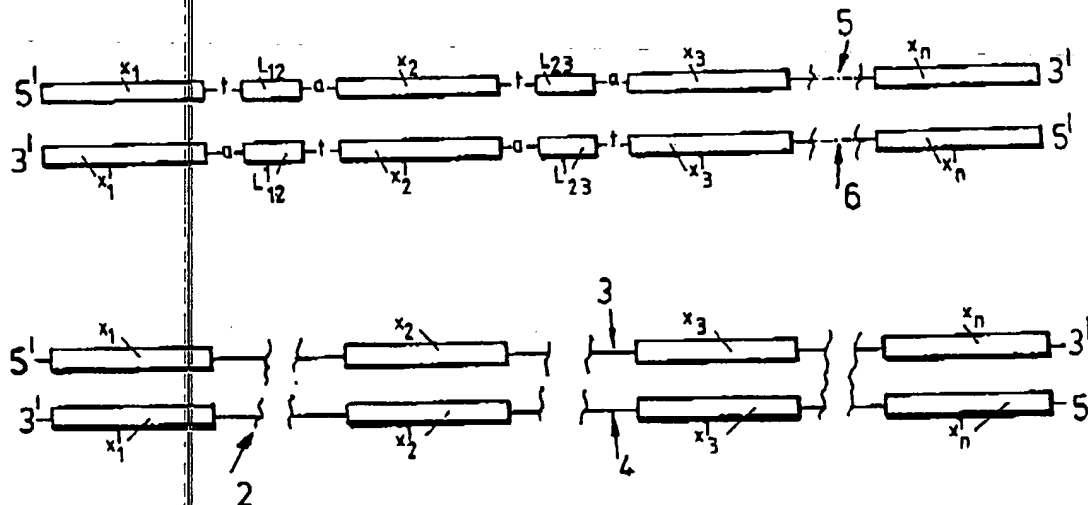
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(54) Title: METHOD OF GENERATING NUCLEIC ACID HYBRIDS FOR MUTATION ANALYSIS



(57) Abstract

A method of producing a hybrid DNA molecule allowing the assembly of sequences x_1, x_2, \dots, x_n where n is greater than or equal to 3 (e.g. give sequences) from diverse locations into a hybrid molecule for the purpose of mutation analysis. The method comprising the steps of: (1) providing in a single reaction mixture: (a) the sequences x_1, x_2, \dots, x_n and their complementary sequences x_1', x_2', \dots, x_n' , to be assembled into the hybrid molecule; (b) for each pair of complementary sequences defined in (a) a respective pair of PCR primers each having a priming sequence and which are such that the primers hybridising to the 3' ends of any two sequences (x_i, x_{i+1}'), where i is 1 to $(n-1)$, have specifically complementary linker sequences; (2) effecting a first stage PCR reaction in which those primers provided with linker sequences are present in limiting concentrations; and (3) effecting a second stage PCR reaction using a single pair of primers one of which provides the 5' end of the sense strand and other of which provides the 3' end of the anti-sense strand of the required hybrid molecule; whereby said hybrid molecule is generated.